# TAY-SACHS DISEASE AND RELATED DISORDERS: FRACTIONATION OF BRAIN *N*-ACETYL-β-HEXOSAMINIDASE ON DEAE-CELLULOSE

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## 1. Introduction

Following the demonstration by Robinson and Stirling [1] of two hexosaminidase components, A and B, in human spleen, similar components have been found in all human tissues investigated [2, 3] and, with one exception [4], it is now well-established that patients with early-infantile $G_{M_2}$-gangliosidosis* are totally deficient in hexosaminidase A [3, 4, 6]. However, the recognition of closely related variants has led to some confusion when the clinical term "Tay-Sachs disease" is used to designate $G_{M_2}$-gangliosidoses; a note on their nomenclature is therefore included.

In the present study, the separation of hexosaminidase components of post-mortem brain grey matter by gradient elution from DEAE-cellulose is described. The patterns obtained in five cases of $G_{M_2}$-gangliosidosis were compared with four controls. The controls all had a very similar enzyme pattern; two major peaks of activity, corresponding to components A and B reported in previous studies [1], were clearly separated from several smaller intermediate fractions. The pattern in each of the five cases differed from the controls, and three types could be distinguished.

* The ganglioside nomenclature used in this report is that of Svennerholm [5].

## 2. Materials and methods

### 2.1. *Materials*

Specimens of frontal cortex were frozen with solid carbon dioxide several hours after death and stored at −20° until examined.

### 2.2. *Identification of gangliosides*

Gangliosides in chloroform-methanol (2:1, v/v) extracts were separated by thin-layer chromatography on silica gel G with chloroform-methanol-1.4 M $NH_3$ (55:40:10, by vol.). Hexosides were separated with chloroform-methanol-water (70:30:5, by vol.).

### 2.3. *DEAE-Cellulose chromatography*

The method was based on that used by Robinson, Price, and Dance [7] for rat kidney enzymes. Supernatant (2 ml) from a 5% w/v homogenate of grey matter in phosphate buffer (10 mM $Na_2HPO_4$ adjusted to pH 6.0 with phosphoric acid) was loaded onto a column (28 cm × 1.5 cm i.d.) of Whatman DEAE-cellulose, type DE52 (W. and R.Balston Ltd) equilibrated with the phosphate buffer at 4°. A linear NaCl gradient in the same buffer was immediately applied and 5 ml fractions were collected at a flow rate of 12 ml/hr.

### 2.4. *Starch-gel electrophoresis*

The conditions were those of Robinson, Price and Dance [7].

2.5. *Assay of hexosaminidase activity*

The method was a modification of that of Leaback and Walker [8]: enzyme solution (100 $\mu$l) was incubated with 3 mM 4-methyl-umbelliferyl 2-acetamido-2-deoxy-$\beta$-D-glucopyranoside (Koch-Light Laboratories Ltd) in sodium phosphate-citrate buffer, pH 4.5 [9] (100 $\mu$l) at 37° for 20 min; 0.25 M glycine adjusted to pH 10.4 with NaOH (1.0 ml) was then added and the fluorescence measured in an Aminco-Bowman spectrofluorimeter, excitation, 360 nm; emission, 440 nm.

## 3. Results and discussion

Hexosaminidase components A and B separate as two broad bands on starch-gel electrophoresis of homogenates of normal brain. However, a more complex pattern was obtained after fractionation by gradient elution from DEAE-cellulose, as shown in fig. 1 and summarized in table 1. The hexosaminidase component defined as A in the electrophoretic separation [7] is equivalent to the last peak of activity eluted from the DEAE-column.

The presence of several minor peaks of activity was also indicated by iso-electric focussing [4]. Since hexosaminidase A can be degraded by neuraminidase to a form electrophoretically identical to hexosaminidase B [1], it is possible that these minor peaks represent hexosaminidase components with intermediate numbers of *N*-acetylneuraminic acid residues.

The clinical and chemical findings in the five cases studied are summarized in table 2. The resulting classification into three types was fully supported by studies on the brain hexosaminidase patterns as shown in fig. 1 and table 1, and confirms recent reports of the occurrence of these variants [4, 10].

The finding that the hexosaminidase activity can be resolved by DEAE-cellulose chromatography in-

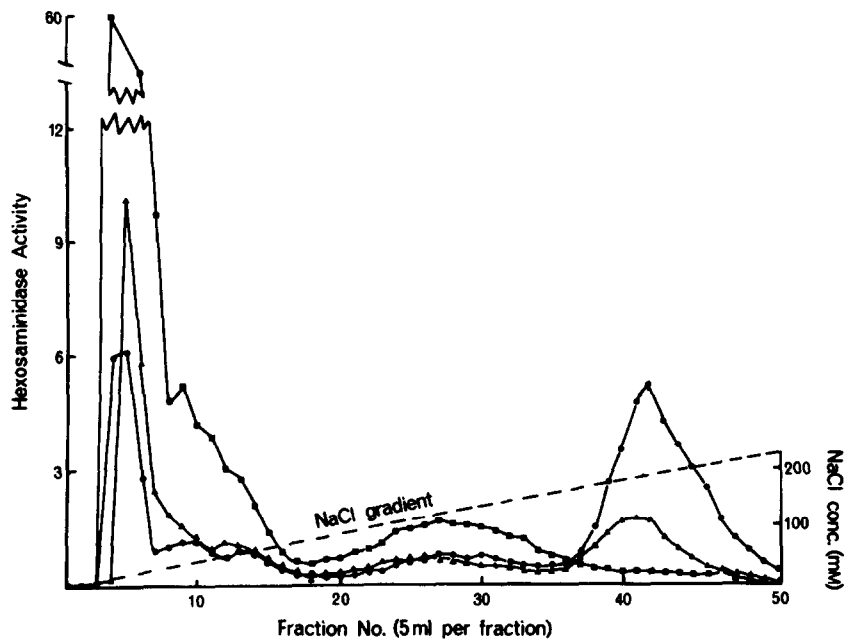


Fig. 1. Fractionation of brain hexosaminidase on DEAE-cellulose. ●—● Control; ■—■ Case 1, early-infantile $G_{M_2}$-gangliosidosis; ▲—▲ Case 5, late-infantile $G_{M_2}$-gangliosidosis. All fractions from Case 4, early-infantile $G_{M_2}$-gangliosidosis, were inactive. Hexosaminidase activity is expressed as nmoles substrate hydrolysed per hour per 100 $\mu$l of individual fractions in the standard assay system.

Table 1
Fractionation of *N*-acetyl-β-hexosaminidase components by DEAE-cellulose chromatography.

| Fraction numbers | Equivalent component by starch gel electrophoresis | Equivalent component by isoelectric focussing | Relative distribution of components | | |
|---|---|---|---|---|---|
| | | | Controls | $G_{M_2}$-gangliosidosis* Cases 1, 2, 3 | Case 5 |
| 4 to 6 | B | Component with isoelectric point pH 7.4 | Major peak | Greatly elevated | Slightly elevated |
| 8 to 15 | B | – | Two minor peaks | Incompletely resolved from first major peak | As for controls |
| 20 to 35 | B | – | Broad, low peak with indication of more than one component | Same position as for controls but about double the activity | As for controls |
| 36 to 50 | A | Component with isoelectric point pH 5.0 | Major peak | Absent | Same position as for controls but about one third of the activity |

* No activity found in any fraction from Case 4.

to more than the two components seen after electrophoretic separation could be important in diagnosis as well as in the elucidation of possible molecular relationships between these components. It might enable new variants to be recognised and should increase the reliability of the diagnosis in cases of late infantile $G_{M_2}$-gangliosidosis in which the deficiency of hexosaminidase A is incomplete (see fig. 1).

In addition to the three types of $G_{M_2}$-gangliosidosis listed in table 2, two further variants have been

Table 2
Summary of clinical and biochemical findings.

| Case number | Clinical presentation | Lipid stored | Suggested systematic nomenclature* |
|---|---|---|---|
| 1, 2, 3 | Early-infantile onset. Conventional symptoms of Tay-Sachs disease | $G_{M_2}$ | $G_{M_2}$-gangliosidosis, Type $A_OB_H$ |
| 4 | Early-infantile onset. Conventional symptoms of Tay-Sachs disease | $G_{M_2}$ and globoside | $G_{M_2}$-gangliosidosis, Type $A_OB_O$ |
| 5 | Late-infantile onset. Symptoms similar to case described by Volk et al. [13] | $G_{M_2}$ | $G_{M_2}$-gangliosidosis, Type $A_LB_N$ |

* see Section 4.

described. The first of these was reported by Sandhoff [4] to be clinically identical to typical cases of Tay-Sachs disease, to store ganglioside $G_{M_2}$ in nerve tissue, and to have normal proportions of hexosaminidase components A and B, though both were elevated. The other variant was reported by Suzuki [11] as a case of "juvenile $G_{M_2}$-gangliosidosis". Although the total hexosaminidase activities of brain, liver, and spleen were normal, a study of the relative activities of components A and B has not yet been reported. This case had many features in common with the late-infantile variant and may also be found to have a similar partial deficiency of hexosaminidase A. We can report a similar case from this hospital of a girl whose symptoms first appeared at the age of 9 years and who died aged 14 years. Although only formalin-fixed brain was available for analysis, storage of $G_{M_2}$-ganglioside was clearly demonstrated.

## 4. A note on the nomenclature of the gangliosidoses

The generally accepted classification of the gangliosidoses according to the nature of the lipid stored was first proposed by Suzuki and Chen [12]. They recognised that subdivision might become necessary as knowledge advanced and, with the recent findings and possible further discovery of specific enzyme abnormalities in closely related variants of $G_{M_2}$-gangliosidoses, such sub-division is now required. O'Brien [10] has referred to these variants as "Types 1, 2 and 3" but it is desirable to employ a comprehensive system of nomenclature from which the nature of the specific enzyme defect is apparent. We therefore propose the modification shown in the right-hand column of table 2, where A and B refer to the two major components of hexosaminidase and subscripts show the nature of the abnormality: H(high), N(normal), L(Low), and O(no activity).

The unusual case reported by Sandhoff [4] would be designated as "$G_{M_2}$-gangliosidosis, Type $A_HB_H$". A similar system of nomenclature could be used to define other conditions involving varying deficiencies of a multi-component enzymic activity.

A comment on the use of the clinical designation "Tay-Sachs disease" is necessary in view of the demonstrably different conditions to which it is now applied; three distinct uses of the term are found in the literature: i) for all cases of $G_{M_2}$-gangliosidosis; ii) for early infantile $G_{M_2}$-gangliosidosis; iii) for only those cases of early infantile $G_{M_2}$-gangliosidosis in which hexosaminidase component A is absent and component B present. Volk and his colleagues [13] concluded that late-infantile $G_{M_2}$-gangliosidosis is clinically distinct from typical cases of Tay-Sachs disease and this distinction applies equally to juvenile $G_{M_2}$-gangliosidosis [11]. Use (i) above is therefore inappropriate. Since those cases lacking both A and B components of hexosaminidase are clinically identical with cases lacking only component A, the clinical term "Tay-Sachs disease" applies equally to both and will certainly continue to be used for these conditions, as in (ii) above. The differences between these variants are made apparent by the use of the systematic nomenclature suggested above.

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